



Institute of Clinical Pathology  
and Medical Research

# Laboratory testing at the State Level

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# Why do laboratory testing for influenza?

- Many pathogens cause an ‘influenza-like illness’
- Many pathogens cause outbreaks
- Allows appropriate infection control and public health management
- Determines the use of correct treatment and cessation of incorrect treatment
- Surveillance and vaccine determination
- Possible pandemic

# Principles in investigating an 'ILI'

- Appropriate respiratory tract samples
  - using the correct swabs
  - collected at the right time in the clinical illness
  - collect safely
- Quick and reliable transport to the laboratory
- COMMUNICATE! Tell the laboratory what is required
- Interpret the results correctly for the patient, and for public health

# Laboratory confirmation

- 1) Isolation of influenza virus by culture
- 2) Detection of influenza virus by nucleic acid testing
- 3) Detection of influenza antigen (IF, EIA)
- 4) Seroconversion or fourfold rise in antibody titre to influenza virus (or single high titre)
- 6) Positive result in a rapid antigen or point of care influenza test

# Diagnostic tests

	<i>sensitivity</i>	<i>turnaround time</i>
<i>culture</i>	+++	4-5 days
<i>rapid culture</i>	+++	1-4 days
<i>immunofluorescence</i>	+++	2-4 hours
<i>nucleic acid testing</i>	++++	1-2 days (RT-PCR 4-5 hrs)
<i>point-of-care tests</i>	++	15-30 minutes
<i>serology</i>	+++	1-3 weeks

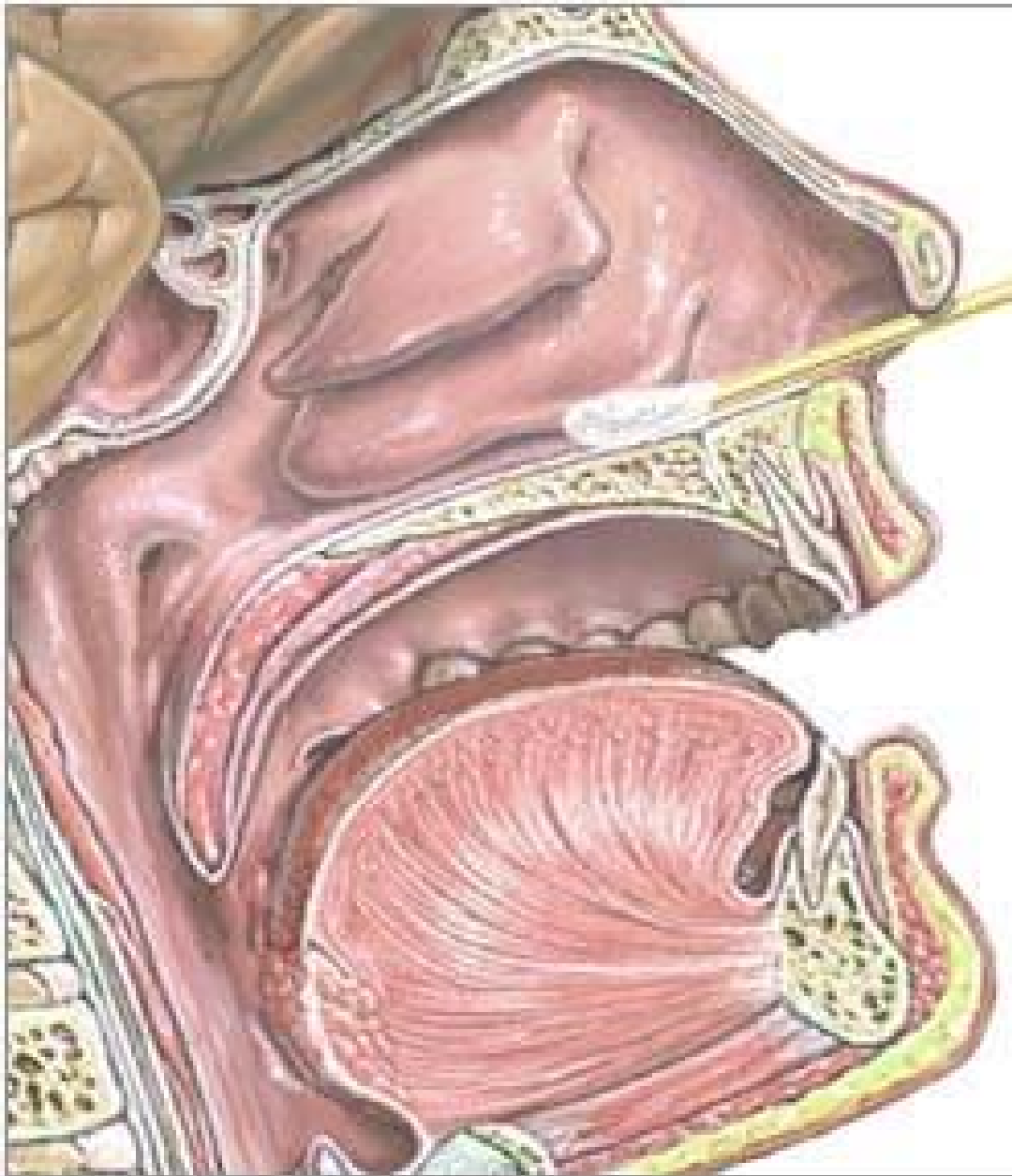
# Specialised Laboratory Testing

- specialised analyses
  - drug resistance
  - strain typing and antigenic variation
  - vaccine assessment
  - non-human viruses etc
- correlation with epidemiology
  - reporting to PHU
  - what is circulating in the community
  - outbreaks

Laboratory diagnosis is dependent on the quality of specimens collected from the respiratory tract during the acute phase of the illness

# Samples to collect in a pandemic

- Combined nose and throat swab
- Nasopharyngeal aspirate (<3 years)
- Single nose swab, nasopharyngeal swab, throat swab, nasal wash, sputum are less satisfactory
- Bronchoalveolar lavage
- Blood, CSF, faeces, tissue
  
- Depends on where the patient is, and what tests will be done



A sterile swab is passed gently through the nostril and into the nasopharynx

# Transport

- Transport combined N/T swabs at room temperature for PCR and IF
- Transport at 4°C for culture
- Depending on location either double bag in dedicated esky OR
- Package as Category B (diagnostic specimens) - IATA Packing Instruction 650. Package and air waybill states: 'Biological Substance Category B, packed in compliance with IATA Packing Instruction 650.'

<b>Location</b>	<b>Sample type</b>	<b>Transport</b>
General practices	combined N/T serology	refer
Hospitals (large to small)	all sample types	test or refer
Fever clinics	combined N/T (or none) serology	refer (?POC)
Pathology laboratories	combined N/T serology	test or refer
Mortuaries	tissue	refer

# Facilities undertaking influenza diagnosis in NSW

- WHO Network - Collaborating Centre and National Influenza Centres (Pathwest, VIDRL and ICPMR)
- PHLN laboratories (Public Health Laboratory Network - ICPMR and SEIALS)
- 'Hub' laboratories
- Large hospital laboratories
- Small hospital laboratories
- Private laboratories
- Other sites eg fever clinics, A&E departments
- (veterinary, research laboratories)

# Influenza testing strategies

- ‘routine’ investigation of ILI
- outbreaks
- ‘out of season’ or ‘unusual exposure’
- pandemic

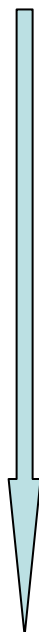
# Investigations specific for a pandemic strain (eg. H5N1)

- Nucleic acid testing (PC2 level)
  - H5-specific
- Virus culture (PC3 level)
- (antigen detection, rapid tests, serology)
- (sequencing)

# Investigations not specific for a pandemic strain (eg. H5N1)

- Nucleic acid testing
  - All influenza subtypes
  - Seasonal influenza
  - Other respiratory pathogens
- Antigen detection (IF, EIA, rapid tests)
  - Influenza A (H3 and H1) and B
  - Other respiratory pathogens
- Serology
- Antiviral resistance testing (N1 and N2)

# Current pandemic alert level

Period	Phase	Description	Main strategy
Inter-pandemic	0	No animal infection (with subtype that has caused human disease in the past)	<p>Containment</p>  <p>Maintenance of essential services</p>
	1	Animal infection: low human risk	
	2	Animal infection: substantial human risk	
Pandemic alert	3	<b>Human infection with novel subtype: no spread or at most rare instances</b>	
	4	Human infection with novel subtype: small clusters, limited human to human transmission	
	5	Human infection with novel subtype: larger clusters, substantial pandemic risk	
Pandemic	6	Pandemic: increased and sustained transmission in the general population	

# Testing strategies in a pandemic (ICPMR)

- Pre-pandemic phases (Overseas 3, Aus 0)
  - Usual testing for respiratory viruses
  - Influenza H1-16 PCR with H5-specific primers and/or
  - H5-specific PCR
  - (serology, POCT, sequencing)

# Testing strategies in a pandemic (ICPMR)

- Early pandemic phases (Overseas 3-5, Aus 3-4)
  - H5-specific PCR or influenza H1-16 PCR with H5-specific primers
  - if negative - usual testing for respiratory viruses
  - (culture at BSL 3 and transport to WHO CC, antiviral resistance testing)
  - (serology)

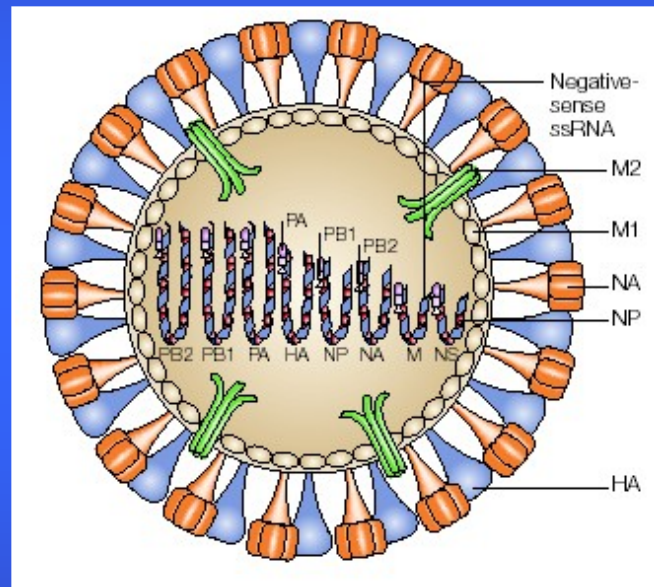
# Testing strategies in a pandemic (ICPMR)

- pandemic phases (Aus 5-6)
  - H5-specific PCR
  - Influenza-specific, but non pandemic strain-specific, assays (eg. IF, point of care)
  - (culture at BSL 3 and transport to WHO CC, antiviral resistance testing)
  - Serology
  - If negative, consider testing for respiratory viruses
  - No testing

# Laboratory activities 2006-07

- Evaluation of nucleic acid testing and POC tests protocols (NHMRC and PHLN)
- Neuraminidase inhibitor resistance testing (WHO, NHMRC and WMI)
- Quality assurance programs - WHO and RCPA
- Specimen collection and POCT training (DoH, NH)
- Practice runs
  - 100 extractions and PCR (ICPMR)
  - Planned laboratory evaluation (PHLN)
- Sequence review for PCR primers and probes (ICPMR)

# Development of National Protocols for the Detection of Influenza A H5N1



## Chief Investigators

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# Nucleic acid testing

- Several H5 primer pairs can be recommended

CDC (dual probe & TQM) PathWest (Ochre and Black)

VIDRL H5 TQM

ICPMR

- Two NA extraction platforms can be recommended
  - Corbett Xtractor/ Sigma chemistry
  - QIAGEN Biorobot/ QIAGEN chemistry

# Rating of Point of care tests

<b>Assay</b>	<b>Virus</b>	<b>Comparative sensitivity</b>	<b>Ease of use</b>	<b>Specimen range</b>	<b>Rating</b>
QuickVue Influenza A+B	A and B	7	3	2	<b>12</b>
ImmunoCard STAT! Flu A&B	A and B	5	3	3	<b>11</b>
Binax NOW Flu A+B	A and B	6	3	2	<b>11</b>
SD BIOLINE Influenza Ag	A and B	6	3	2	<b>11</b>
XPECT Flu A + B	A and B	5	3	3	<b>11</b>
Directigen EZ Flu A + B	A and B	5	3	2	<b>10</b>
DK05-FL-001	A and B	5	2	3	<b>10</b>
SAS Flu A/B dipstick	A and B	5	3	2	<b>10</b>
Actim Influenza A+B	A and B	5	2	3	<b>10</b>
Influ-A & B RespiStrip	A and B	5	2	2	<b>9</b>

# Nursing home outbreaks 2006

Outbreak identifier	Symptom onset date of first case	Aetiology	Number of residents in facility	Total number of cases (suspect + confirmed)	Number of cases in residents	Attack rate in residents	Duration of outbreak (days)^	Time from first case^^ to first POC test	Time from first case^^ to an outbreak being identified
A	18 Jun	Not identified -- not influenza	78	19	18	23%	17	11 days	11 days
B	8 Jul	Not identified -- not influenza	160	10	10	6%	20	4 days	4 days
C	14 Jul	Not identified -- not influenza	130	28	28	22%	19	4 days	4 days
D	23 Aug	Parainfluenza 1	80	12	11	14%	10	2 days	4 days
E	20 Aug	RSV	147	16	13	9%	18	11 days	11 days
F	30 Sep	Influenza A	92	17	13	14%	19	2 days	6 days
G	3 Nov	Influenza A	160	9	0	0%	7	3 days	5 days
Z*	11 Oct	Influenza A	132	77	55	42%	56	? days	21 days

POCT versus IF - sensitivity 71%, specificity 97%

